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Trajectories of genetics, 150 years after Mendel/Trajectoires de la génétique, 150 ans après Mendel

Gene therapy: Myth or reality?

Thérapie génique : mythe ou réalité ?

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ABSTRACT

Gene therapy has become a reality, although still a fragile one. Clinical benefit has been achieved over the last 17 years in a limited number of medical conditions for which pathophysiological studies determined that they were favorable settings. They include inherited disorders of the immune system, leukodystrophies, possibly hemoglobinopathies, hemophilia B, and retinal dystrophies. Advances in the treatment of B-cell leukemias and lymphomas have also been achieved. Advances in vector development and possible usage of gene editing may lead to significant advances over the next years.
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Édition de gène

RÉSUMÉ

Bien qu'encore fragile, la thérapie génique est devenue une réalité. Un bénéfice clinique a été obtenu au cours des 17 dernières années dans un nombre limité de conditions médicales pour lesquelles des études physiopathologiques avaient déterminé qu'elles étaient favorables. Il s'agit des troubles héréditaires du système immunitaire, des leucodystrophies, éventuellement des hémoglobinopathies, d'hémophilie B et des dystrophies rétiniennes. Des progrès dans le traitement des leucémies à cellules B et de certains lymphomes ont également été accomplis. Les progrès dans le développement de vecteurs et la possibilité d'utiliser l'ingénierie génomique ciblée pourront conduire à des progrès importants au cours des prochaines années.
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1. Introduction

Gene therapy has attracted many scientists and clinicians for its potential to fix a disease based on genes. This was initially viewed as a way to correct genetic diseases, but further applications were rapidly considered since genes products can convey cells with characteristics of medical interest to fight cancer or protect from cell degeneration, for example. Ease in gene manipulation, addition of regulatory elements and usage of viruses mostly to drive entry into cells to target made this concept potentially feasible as envisaged in the early 1970s [1].

Nevertheless, it was rapidly perceived, despite a number of unsubstantiated claims, that gene transfer needs to be efficacious to overcome many hurdles. They include targeting of the appropriate cell lineage(s), obtaining a sufficient but not excessive level of gene expression, maintaining its expression over time, avoiding the genotoxic effects of the material if integrated into the cells' genome, limiting/eliminating immune reaction against the vectors and/or the gene's product... This explains, why for many years, the multiple attempts, notably in the area of cancer, failed, leading to skepticism about its future. In addition, the gene therapy strategy has to be based on an appropriate understanding of the pathophysiology of the disease to treat [2].

2. Strategy and vectors

Today, available technologies made feasible both ex vivo and in vivo gene therapy based on the persistence of the transgene either non-integrated or integrated in the genome. The former approach is appropriate to target post-mitotic cells, while the second is necessary to target mitotic cells (such as hematopoietic cells, for instance). Adeno-associated viral vectors (AAVs) are well matched for the first approach. They can infect virtually all cell types and permit to achieve stable transgene maintenance. Such vectors can relatively easily be engineered as non-replicative viruses and produced as very large batches. Inconveniencies rely on the relatively limited size of genetic material that can be delivered and on its immunogenicity. Indeed AAVs naturally infect humans who develop effective immune responses against its components, which can lead to neutralization of efficacy by destruction of transduced cells. Some AAV strains (such AAV8 or 9) do less frequently infect humans, leaving some opportunity for application (see below) [3].

Retroviruses offer the capacity of leading to integration into the genome. Gamma retroviruses, then lentiviruses have been designed to carry the genetic material into the cells and lead to stable integration into the genome(s) [4]. HIV-based lentiviral vectors are particularly attractive since their provirus can integrate both in dividing (like γ retroviral vectors) but also in non-dividing in the G1 phase of the cell cycle [5]. The major drawback of the utilization of retroviral vectors is the semi-random character of their integration, which can cause genotoxicity (see below).

Gene therapy can be considered as a way to add the copy of a gene, to modify a gene (for instance to skip a mutated exon), to inactivate a gene (for instance in the setting of a dominant mutation with a trans negative effect) or to correct a gene mutation based on recombinant technology.

3. Applications

3.1. The hematopoietic system

The first success of gene therapy were achieved around the year 2000 in the very specific field of treating severe inherited T-cell immune deficiencies by ex vivo gene transfer into hematopoietic stem (HS) or progenitor cells (C). Several reasons account for that. HSCs are accessible while the prospect of modifying HSCs should provide lasting benefit. In addition, T cells are known to be long lived and the analysis of rare revertant cases from severe combined immune deficiencies – sort of natural gene therapy – showed that precursor T cells can expand, providing demultiplication of gene transfer. Two diseases, severe combined immunodeficiency X1 (SCID X1) and adenosine deaminase deficiency (ADA), have thus been treated with success by using γ retroviral-mediated gene transfer into hematopoietic progenitor cells [6,7]. The efficacy has now been found sustained for 17 years since patients exhibit close to or normal T lymphocyte counts and function, enabling them to live normally. Nearly 100 patients have now been successfully treated world-wide [8–10]. Nevertheless, this success was tempered by the occurrence in the *SCID X1* gene therapy trials (five cases) and in other trials of cases of leukemia [11]. The latter originated from oncogene transactivation by the viral enhancer from the long terminal repeat (LTR) following proviral integration within the oncogene locus. These unanticipated genotoxic events led to halt clinical trials. Once the mechanism was understood, a new generation of vectors was produced, in which the viral LTR was deleted and instead an internal promoter was used, the so-called self-inactivated (SIN) vectors. These SIN vectors have now been used with success and safety with a follow up reaching more than eight years for several diseases including SCIDX1 [12,13].

The first successes of gene therapy were obtained in a favorable setting because of the selective advantage provided to the transduced cells. Utilization of vectors that are more potentially able to transduce HSCs, i.e. lentiviral vectors, have now been used with success to treat additional genetic diseases, including the Wiskott–Aldrich syndrome, another form of primary immunodeficiency [14,15] and then leukodystrophies, for which gene addition in the monocyte cell lineage was of interest [16,17]. The extension of indications to many more genetic diseases of hematopoiesis is being envisaged; it includes several primary immune deficiencies, but also Fanconi anemia or genetic disorders of hemoglobin (β-thalassemia and sickle cell disease).

The latter represents a formidable challenge, as transgene (β-globin) expression needs to be restricted to the erythropoietic lineage. Introduction of the regulatory locus control region makes it feasible. First trials have been initiated with promising preliminary results [18]. An

alternative strategy, such as promoting the expression of fetal hemoglobin by inactivating a regulatory transcription factor, appears as particularly elegant.

3.2. Other applications in the field of inherited diseases

The liver is a potentially good target for in vivo (intravenous) AAV vector-based gene therapy. It has been primarily used to treat hemophilia B factor (factor IX deficiency). While the first attempts failed because immune responses of pre-immunized recipients to the AAV2 virus eliminated transduced hepatocytes, the recent choice to use instead AAV8 in naïve recipients led to a sustained ($\geq$ 4 years) persistence of FIX plasma expression, sufficient to avoid most of the clotting factor replacement injections [19,20]. No side effects were observed. Another useful application of the same technology consists of an intraocular (subretinal) injection of AAV vectors containing a normal copy of genes mutated in various forms of inherited retinal dystrophies. These attempts were found safe, did not generate immune responses, and led to some improvement in sight that can be sustained at least for some years, provided that patients are not treated too late when retinal cells to be targeted are lost [21–23]. Many more projects based on AAV or lentiviral vectors are being tested to treat a variety of inherited disorders affecting brain (mucopolysaccharidosis), muscle (myopathies), or liver (several metabolic diseases) [24]. They have not yet reached the stage where clinical benefits have been demonstrated, in part because targeting of diseased cells (brain and muscle, but also lungs) remains a serious challenge.

4. Gene therapy of cancer

After years of failures, first convincing results were achieved over the last five years with a strategy consisting

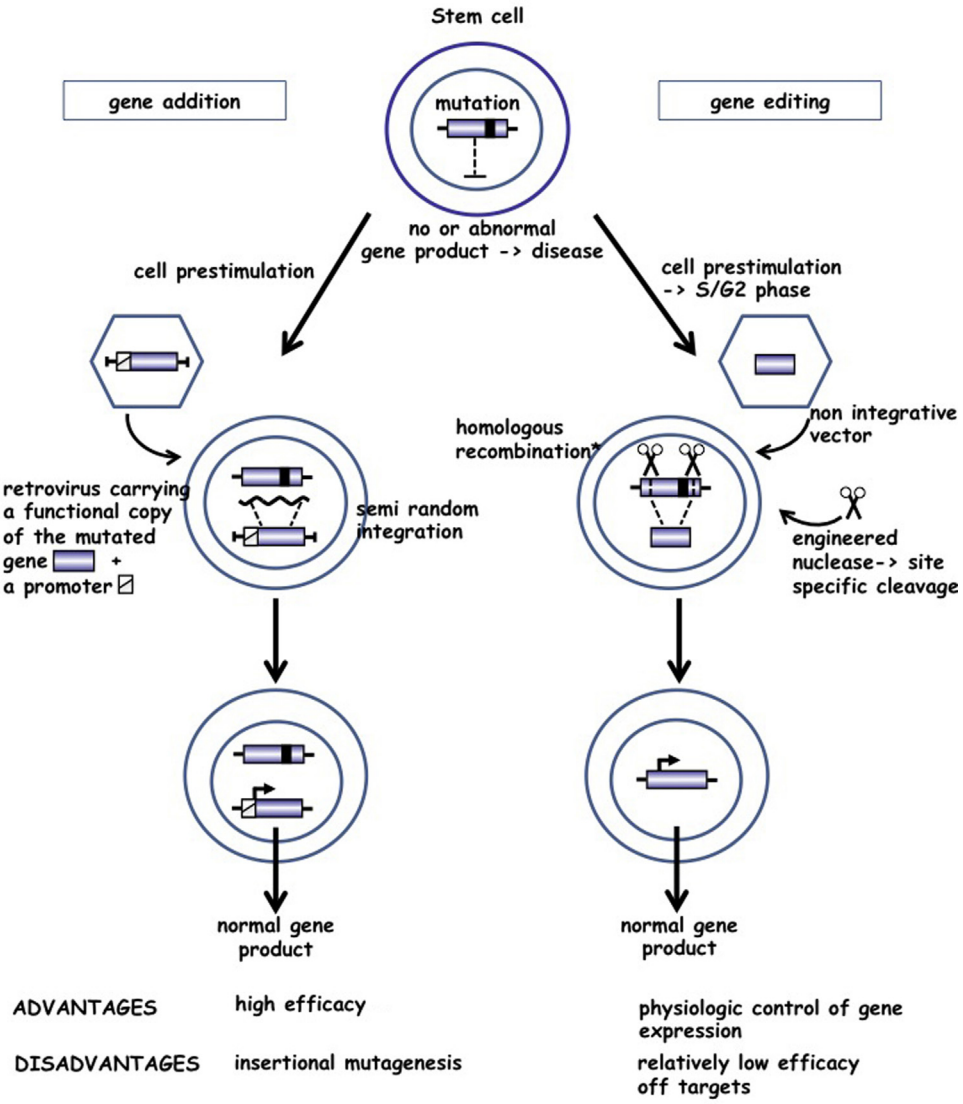

Fig. 1. Two strategies for gene therapy: gene addition and gene editing.

of harnessing T lymphocytes with a chimeric receptor capable of recognizing a membrane protein at the surface of cancer cells and activating these T cells to kill the target cells. Such chimeric antigen receptors (CAR) have been engineered based on a variable domain of immunoglobulin (recognition unit) coupled with potent activation transducing domains. This technology has been successfully applied to the treatment of B-cell leukemias and lymphomas by targeting an intrinsic membrane B-cell molecule (CD19). Lentiviral vectors were designed to ex vivo transduce the patients' T cells. Several clinical trials have demonstrated that this approach can induce remission in patients with refractory disease [25,26]. The loss of B cells can be compensated by immunoglobulin supplementation. Although some adverse events can be generated by the potency of the inflammation reaction, this strategy is sufficiently promising to enable several pharmaceutical companies to investigate now its development, with the additional aim to design CAR able to recognize molecules on solid tumors too.

5. Further prospects

A number of challenges remain ahead of the development of gene therapy for it to become some form of standard treatment. Large-scale manufacturing of vectors remains an issue, although advances have been readily achieved. New technologies, particularly based on targeted gene editing (Fig. 1) mediated by engineered nucleases, appear particularly appealing. The successive generation of such modified enzymes: Zn finger nucleases, Talen and now RNA guided Cas 9 make it today feasible to very specifically target genome modifications [27]. A first usage will be gene inactivation by letting targeted DNA breaks in a given gene be repaired with errors by the non-homologous DNA end joining (NHEJ). This has entered clinical testing by disrupting with adapted Zn finger nucleases both alleles of the HIV CCR5 receptor encoding genes in T cells to protect them from infection [28]. The safety and the long-term persistence of such cells have been demonstrated, although it will be needed to significantly increase the number of targeted cells for it to be effective. The very same strategy is being experimentally tested to inactivate the expression of a mutated exon – for instance – in the dystrophin gene associated with myopathies [29] or to extinguish the expression of molecules involved in unwanted immune responses, such as HLA antigens, to avoid the rejection of potentially "universal" corrected cells as envisaged with engineered anti-cancer T cells.

The same technology may be used to initiate homologous recombination at a desired locus to replace a mutation by a wild-type sequence. Proofs of principles of success have been achieved in cell lines [30] and animal models of inherited diseases, such as SCID X1 tyrosinemia [31] or urea cycle deficiency [32]. Still, the frequency of cell editing and the issue of potential off targets (i.e. non-locus-specific recombination) need to be addressed. Some have advocated that one day this technology could be used to repair germ line mutation in embryos. Although technically it may become feasible, the raised ethical questions, knowing in particular that pre-implantation diagnosis can avoid the birth of affected children, represent a barrier that is likely not to be trespassed.

6. Conclusion

Combination of gene therapy with stem cell technology may widen its scope once the latter will be mastered up to the point of medical application of gene therapy to medicine. In any case, it should remain clear that gene therapy will never be more than one additional strategy in the armamentarium of therapeutics, but this will be a significant achievement!

Disclosure of interest

The author declares that he has no competing interest.

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